THE BLUEBERRY RHIZOME: IN VITRO CULTURE[1]

W. G. Barker and W. B. Collins
Research Station, Canada Department of Agriculture, Fredericton, New Brunswick

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Abstract

The successful in vitro culturing of blueberry (*Vaccinium angustifolium* Ait). rhizome apices is reported. A variation in sensitivity to short light exposures is noted that may be seasonal. In spring the organ appears less light sensitive. Later no exposure is tolerated without response. In dark culture of spring-gathered rhizomes, the diageotropic habit continues with the evolution of scale leaves separated by long internodes. Exposure to a 16-hour light/dark cycle, however, causes the apex to elevate and induces changes in habit that are manifested by the shortening of the internode and the expansion of the scales into green leaves. Elongating growth essentially ceases. Histological studies suggest that a disruption of the apex follows light exposure. Necrotic cells appear behind the dome, and the meristem decreases in size and changes shape even as it rapidly evolves foliar initials. Apparently the meristem rapidly loses function.

The method is offered as a technique suitable for the controlled study of rhizome physiology and development.

Introduction

The growth and development of underground stems is imperfectly understood. The rhizome of the lowbush blueberry typically grows beneath the soil surface and may sporadically form a leafy shoot either by conversion of the apical meristem or from a bud on the axis. Whether the aerial shoot develops in response to some external signal or is accountable to some endogenous stimulus is not known. Perhaps the most searching study of diageotropism, where the organ assumes a horizontal posture, is that of Bennet-Clark and Ball (2) which suggests a mobile growth substance balanced by a less mobile antisubstance. The interplay of these two controls the posture of the rhizome.

In the economy of the lowbush blueberry the rhizome is a most important organ. Lateral spread of the plant occurs through the aggressive development of these structures. In commercial cultivation, it makes possible the system of pruning by periodic burnings. A satisfactory crop is obtained normally from flower buds set in the year of the burn on shoots that emerge either from the rhizome or from the unburned bases of aerial shoots. The berries are obtained the year after the burning operation (9). In succeeding years the crop is reduced (3) because of increased vegetative growth with a decreased flower potential. The harvesting of succeeding crops becomes increasingly expensive.

Vegetative propagation of blueberries is possible by making cuttings from almost any material (first-year wood, second-year wood, rhizomes) with a high rate of survival. There is, however, difficulty in promoting the rapid spread of established cuttings to form a commercially acceptable stand. Clearly the problem relates to the formation and subsequent development of rhizomes (9). Since, in addition, the blueberry (*Vaccinium angustifolium* Ait.) is a heterogenous species (3) with divergent clonal characteristics, including a varied ability to form rhizomes, the problem is crucial if propagation and establishment of selected material is to be considered.

[1]Contribution No. 110.

The in vitro culture of rhizomes does not constitute a prominent part of the tissue culture literature. Indeed, Kandler (7) failed to obtain successful cultures from several sources. On the other hand, portions of rhizomes, i.e., artichoke tissue (4), potato stolon nodes (1), and crab grass nodes (6), have been grown successfully.

The present researches include an effort toward understanding the developmental morphology and the physiology of the blueberry rhizome. This initial paper outlines a successful cultivation of isolated blueberry apices in vitro and offers a technique suited to the precise study of these peculiar organs. Additionally, it discusses their sometimes extreme sensitivity to light and details histological changes to the apex of cultured rhizomes taken suddenly from the dark to intense light.

Materials and Methods

In the first attempt at organ cultures, the rhizome apices were gathered in full daylight from clones growing in a sawdust pit. They were brought to the surface, identified, cut into apical 4- to 6-in. lengths, wrapped immediately in moist paper towels, and the whole enclosed in light excluding foil paper. Exposure to daylight was in the order of 30 seconds. Subsequently they were placed in a solution of 12% Javex (7% available chlorine), to which a drop of Tween 20 had been added for 12 minutes in the dark. In a room lighted with one 7-w fluorescent bulb, they were placed by aseptic methods on White's (10) medium (it was not necessary to supplement this with growth substances) and restored at once to the dark, a light exposure of a further 30 seconds. The cultures, in 4-oz screwcapped bottles, were then stored in the dark at 60° F or were placed in a growth chamber under a 16-hour day with a 60° F night temperature deviating to 74° F under the lights. Cultures were removed from the dark periodically for examination and were restored to the dark.

Following 10 days in vitro, 10 cultures that were growing rapidly in the dark were placed under a 16-hour day length, 2 ft below two fluorescent 96-in. Northern Electric Gro lux tubes interspersed with five 100-w incandescent bulbs. Apices were selected and fixed from the dark and at 3-day intervals from under the lights. Fixation was accomplished in formalin:acetic acid:water, and slides stained in Heidenhain's hematoxylon after a ferric chloride mordant were prepared according to the paraffin method of Johansen (5).

In subsequent studies, a safe light (8) was used during both digging and culturing procedures. Finally, the safe light was only used at minimal intensity during the aseptic culturing operation.

Results

Best growth was obtained on unamended White's (10) medium, and 3-indoleacetic acid and like compounds retarded development. The studies reported herein, therefore, refer to cultures on White's medium without supplements.

In the first attempt, growth was obtained at 60° F in the dark. The development consisted of a continued elongation of the axis with the maintenance of

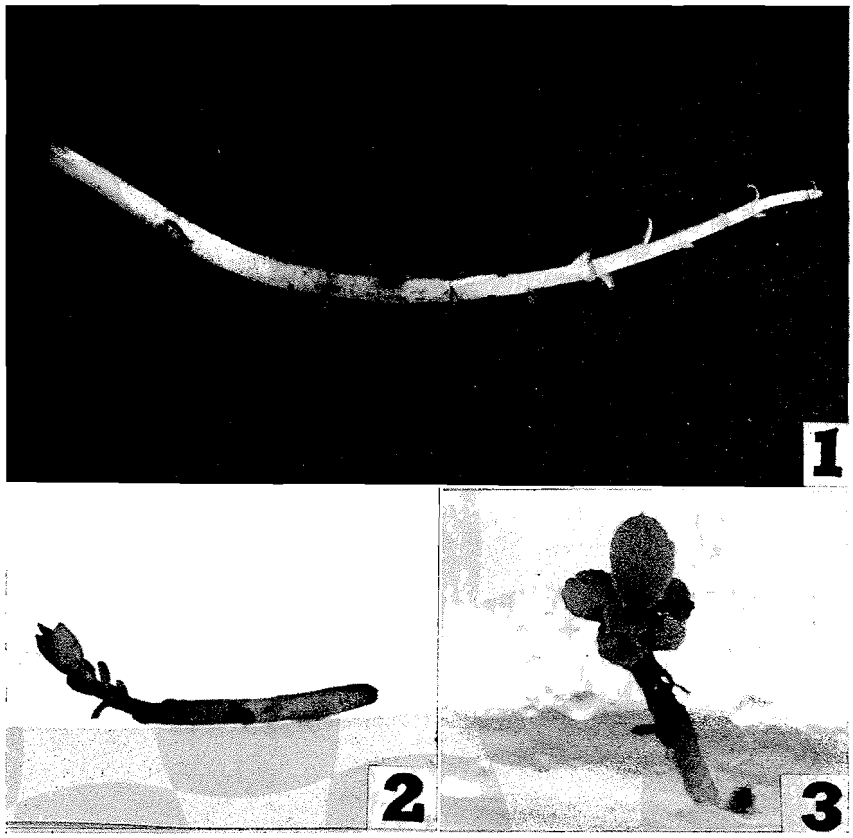


FIG. 1. Blueberry rhizome maintained in dark on White's medium. Note the long internodes and minute scale leaves. ×2

FIG. 2. Blueberry rhizome after 3 weeks exposure to a 16-hour day on White's medium. Note the short internodes, enlarged leaves, and elevation of the apex. ×2

FIG. 3. Blueberry rhizome after 5 weeks exposure to a 16-hour day on White's medium. Note the marked expansion of leaves arranged as a rosette. ×2

the diageotropic habit (Fig. 1), the evolution of scale leaves, and the attenuated habit with long internodes. The color was a cream shade of white. Essentially, the activity of the rhizome appeared uninterrupted by the culturing. The organs rapidly grew to the wall of the container and were in good condition 6 weeks after the culture's initiation, when the last was examined. Cultures moved to the light for inspection and then restored to the dark became vegetatively geotropic and ceased elongating growth.

Inherent bacterial and fungal contaminants were plentiful, limiting the number of cultures that were entirely aseptic.

Those cultures that were stored in a growth chamber under a 16-hour day responded to the light by the rapid development of green pigmentation. The apical portion turned upward and growth continued with markedly reduced internodes (Fig. 2). This figure also suggests the aspect of those

cultures grown in the dark, exposed to light, and restored to the dark, although the leafiness was not manifest in the dark-restored cultures. Eventually, in the long-day cultures, small green leaves appeared that were intermediate in size between rhizomal scale-leaves and blueberry foliage. Where growth continued, the leaves formed a rosette about the upturned axis (Fig. 3). Such cultures survived for 90 days but made no further growth and produced no lateral shoots.

As part of this study, 10 rapidly growing cultures were removed from the dark and placed under a mixture of fluorescent and incandescent bulbs in the laboratory. Apices were fixed from diageotropically growing rhizomes in the dark (Figs. 4 and 5) and two were removed at 3-day intervals from the light. Figures 6 and 7 typify a rhizome apex following 6 days in the light, and the trend of development (or breakdown) of the apex is demonstrated in Figs. 8 and 9, representative of a rhizome selected 11 days after light exposure.

The apex of the dark grown rhizome shows a normal dicotyledon meristem in good condition (Fig. 5). The attenuated nature of the dark-grown organ is evident from the small number of scale-leaf initials present, and from the extent of the internode (Fig. 4).

In Figs. 6 and 7, the apex, following 6 days in intense light, is shown. The pattern of development has changed (Fig. 6). The apex has apparently checked its elongating growth and has produced a markedly increased number of leaf initials. Figure 7 suggests that the shape of the dome may be changing somewhat and that the size is diminished. There is also evidence of cellular disruption and breakdown. Large vacuolate filled cells are clustering immediately back of the dome.

Figures 8 and 9 suggest that the leafy pattern of the stem is continuing with a decreased plastochrone interval. However, the signs of disruption back of the meristem are accentuated (Fig. 9). Additionally, the apex has now decreased markedly in size and presents in toto an unhealthy aspect (Fig. 8).

Several later attempts at culturing blueberry rhizomes in the late summer or early autumn of 1962 failed because the diageotropic posture was invariably lost, the rhizome tip of the explanted organ elevated, and elongating growth ceased. The sequence of events occurred even when all operations were carried in the dark save the ultimate aseptic handling when the explant was introduced into the culture bottle.

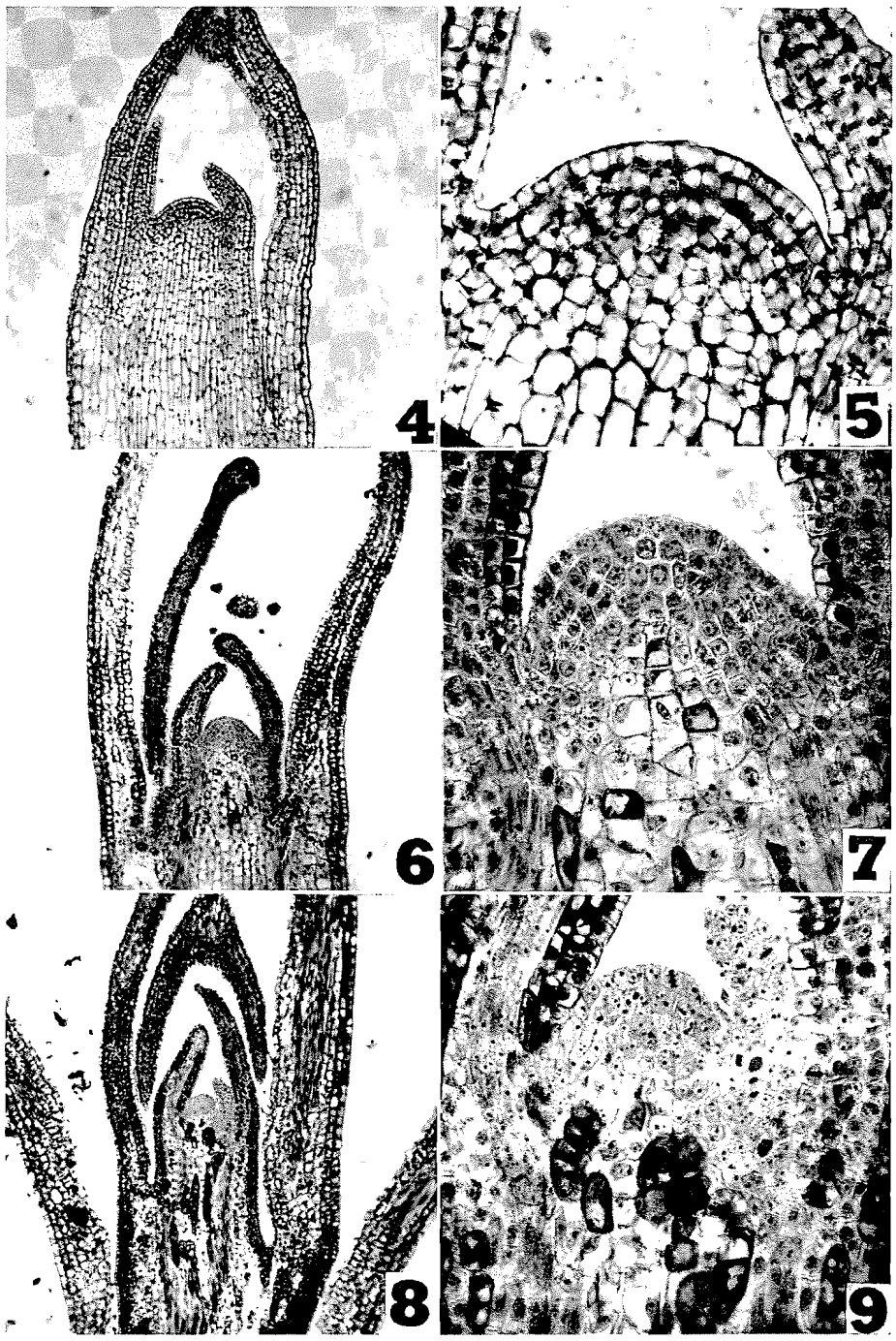
Discussion

Culture of blueberry rhizomes can be established with relative ease and will make satisfactory growth without supplementing the basic nutriment afforded by the formula of White (10). One obstacle has been encountered, however, which seriously limits any wide application of this technique in the study of

FIGS. 4 and 5. Apex and dome of the dark-grown blueberry rhizome in vitro. ×93 and ×465

FIGS. 6 and 7. Apex and dome of dark-grown rhizome following 6 days exposure to light. A marked increase in number of initials is apparent together with a shortened internode. The dome appears diminished and necrotic vacuolate cells can be seen. ×93 and ×465

FIGS. 8 and 9. Apex and dome following 11 days exposure to the light. Numerous leaf initials are present. The diminution of the apex is obvious, as is the breakdown of the meristem and associated tissue. ×93 and ×465



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the blueberry rhizome physiology. The organ is extremely light sensitive and, when struck by light, even light at the safe (8) plant wavelength and at a low intensity, will respond by adopting an ageotropic posture and by terminating shoot extension.

This light sensitivity apparently may be inoperative or inactive under some circumstances since, in the initial experiments conducted in early summer, cultures were established without precaution and subsequently made extensive diageotropic growth in the dark. Separate attempts in late summer of 1962, even when maximum shielding was attempted and when only the very briefest of exposures were permitted, yielded cultures that at once suspended elongating growth with the adoption of the vegetative geotropic posture. No rationale has been made to explain this varying light sensitivity, although it does appear to be a seasonal effect.

In the initial experiments, dark-stored cultures would grow as rhizomes but, if exposed to chronic illumination, they would respond by altering their posture, changing their habit, producing a rosette of green leaves, and then terminating all apparent activity.

The microscope slides prepared to interpret this conversion of habit clearly show the disruption of a normal apical meristem and its reduction to an inoperative unit. This histological evidence fits the observed pattern of gross morphology—a vigorously elongating organ in the dark, a cessation of elgongation, concomitant with negative geotropism upon introduction to the light—and then no further activity.

At this stage, no attempt is made to interpret these phenomena. However, it has been observed that the apex of growing blueberry shoots abort following a similar histological sequence annually. This phenomenon in the shoots may be related to auxin level.

It is apparent that this tissue culture technique offers a strong tool for the manipulation of the blueberry rhizome and for the imposition of highly critical conditions upon it. This nicety of control is eminently suited to the following of changes that accompany the transition of this organ from a rhizome to a leafy shoot.

References

1. BARKER, W. G. 1953. A method for the in vitro culturing of potato tubers. Science, **118**, 384–385.
2. BENNET-CLARK, T. A. and BALL, N. G. 1951. The diageotropic behaviour of rhizomes. J. Exptl. Botany, **2**, 169–203.
3. EATON, E. L. and HALL, I. V. 1961. Blueberry culture and propagation. Research Branch, Can. Dept. Agr., Publ. **754**, 5–21.
4. GAUTHERET, R. J. 1942. Manuel technique de culture des tissus végétaux. Masson et Cie., Paris.
5. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc., New York.
6. JOHNSON, B. G. and BUCHOLZ, K. P. 1961. An in vitro method of evaluating the activity of buds on rhizomes of quack grass (*Agropyron repens*). Weeds, **9**, 600–606.
7. KANDLER, O. 1950. Versuche zur kultur isolierten Pflanzengewebes in vitro. Planta, **38**. 564–585.
8. NITSCH, J. P. and COLETTE NITSCH. 1955. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight growth test of auxins. Plant Physiol. 94–111.
9. TREVETT, M. F. 1956. Observations on the decline and rehabilitation of lowbush blueberry fields. Me. Agr. Exptl. Sta. Misc. Publ. **626**.
10. WHITE, P. R. 1943. A handbook of plant tissue culture. The Jacques Cattell Press, Lancaster, Pennsylvania.